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Effects of UV-B Radiation Levels on Concentrations of Phytosterols, Ergothioneine, and Polyphenolic Compounds in Mushroom Powders Used As Dietary Supplements

Yelena Sapozhnikova,*[,†] William Craig Byrdwell,[‡] Amada Lobato,[§] and Bill Romig[∥]

[†]Eastern Regional Research Center, Agricultural Research Service, USDA, Wyndmoor, Pennsylvania 19038, United States

[‡] Beltsville Human Nutrition Research Center, Agricultural Research Service, USDA, , Beltsville, Maryland 20705, United States

[§]Monterey Mushrooms, Inc., Watsonville, California 95076, United States

[∥]Mid-Atlantic Perishable Commodities Services, Townsend, Delaware 19734, United States

ABSTRACT: Compositional changes of powder dietary supplements made from mushrooms exposed to different levels of UV-B irradiation were evaluated for the bioactive naturally occurring mushroom antioxidant, ergothioneine; other natural polyphenolic compounds, e.g., flavonoids, lignans, etc.; and selected phytosterols. Four types of mushroom powder consisting of white, brown (*Agaricus bisporus*), oyster (*Pleurotus ostreatus*), and shiitake (*Lentinula edodes*) mushrooms from three different treatment groups (control, low and high UV-B exposures) were evaluated. Ergothioneine concentrations found in mushroom powders were 0.4−10.4 mg/g dry weight (dw) and were not appreciably affected by UV-B radiation. No individual polyphenols were detected above 0.1 μg/g. Phytosterols ergosterol (2.4−6.2 mg/g dw) and campesterol (14−43 μg/g dw) were measured in mushroom powder samples. Ergosterol concentrations decreased significantly with the increased level of UV-B treatment for all mushroom powder types, except for white. These results provide some new information on effects of UV-B radiation on these important natural bioactive compounds in mushrooms.

KEYWORDS: *mushroom powder, dietary supplement, phytosterols, polyphenolics, ergothioneine, UV-B radiation*

INTRODUCTION

Mushrooms are consumed worldwide for their organoleptic, nutritional, and medicinal values, providing dietary fiber, carbohydrates, amino acids, vitamins, phenolic compounds, sterols, and other biologically active compounds.[1,2] Numerous health benefits, such as reduced risk of cardiovascular diseases,[2] tumors, and cancer,[3] anti-inflammatory and antiviral resistance, and many others, have been associated with mushroom consumption.[4] Different types of mushrooms are used as food, food ingredients, "functional foods", and dietary supplements.[5] Mushroom powder produced from fresh mushrooms by freeze-drying presumably retains mushroom flavor and nutrients and can be either rehydrated or used as a powder for cooking or as an ingredient in dietary supplement products.[6]

Phytosterols are naturally occurring sterols in mushrooms and other plants, known for their ability to reduce cholesterol levels.[7] Ergosterol is the most abundant and the most studied phytosterol in mushrooms. Following UV light exposure, ergosterol undergoes photolysis to produce previtamin D, which then slowly isomerizes to vitamin D via a thermal reaction.[8,9] The Food and Nutrition Board of the Institute of Medicine recommends dietary reference intakes (DRI) for vitamin D of 600−800 IU (15−20 μg) per day.[10] UV-B irradiation is commonly used to produce high levels of vitamin D_2 in cultivated mushrooms.[9] Dietary supplements made of dried and encapsulated mushroom powder provide high amounts of phytosterols, specifically ergosterol, vitamin D_2, antioxidants, and other bioactive compounds. UV-B irradiated mushroom powder contains up to 6000−8000 IU/g (150−200 μg/g) vitamin D_2. For comparison, one cup (245 g) of vitamin D fortified milk provides 115−124 IU (3 μg) of vitamin D.[11]

Other phytosterols that have been reported in various mushrooms are campesterol and brassicasterol.[9] Stigmasterol, β-sitosterol, stigmastanol, cycloartenol, and lupeol are among other common phytosterols measured in plants,[12] but they have relatively low levels in mushrooms.[9] While the effect of UV light is well characterized for ergosterol in mushrooms, only few reports on the effect of UV radiation on other plant sterols have been found for mushrooms or other food.

Ergothioneine is a natural water-soluble amino acid that has been drawing scientific interest due to its unique antioxidant and cytoprotective properties.[13] It is widely sold in dietary supplement pills and is added to skin care products. Ergothioneine has been reported to be a powerful scavenger of hydroxyl radicals[14] and may protect against oxidative damage.[15] Ergothioneine has been measured in various foods, with the highest reported levels ranging from 0.21 to 2.6 mg/g dry weight (dw) in mushrooms.[16,17] To date, little is known about effects of UV-B radiation on ergothioneine. Search for "ergothioneine" and "UV treatment" in the Web of Science and Scopus databases returned no results, and we are not aware of any published studies to date investigating effects of UV-B treatment in mushrooms (or any other food) on ergothioneine

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levels. Hence, it is not known if UV-B radiation affects ergothioneine concentrations in mushrooms.

In addition to ergothioneine, polyphenolic compounds have been shown to contribute to mushroom antioxidant activity. Polyphenolics are naturally occurring chemicals that belong to diverse classes, including isoflavones, lignans, flavones, stilbenes, flavanones, flavonols, and anthocyanidins, among others. Several studies have found polyphenolic compounds in mushrooms. For example, Liu et al. (2012)[18] measured the flavonol quercetin in wild edible mushrooms from China, while Kim et al. (2008)[19] found naringin, resveratrol, quercetin, naringenin, kaempferol, hesperetin, formononetin, and biochanin A in edible and medicinal mushrooms from Korea. Kim et al. (2008)[19] reported that the antioxidant activity, including free radical scavenging activity of edible and medicinal mushrooms, was well correlated with concentrations of polyphenolic compound measured in mushrooms. It has been reported that UV-B irradiation increased amounts of some polyphenolic compounds, specifically flavonoids naringenin, rutin, and quercetin in tomato fruits[20] and some flavonols and flavonoids in apples,[21] but no reports have been found for mushrooms.

This study intended to fill these gaps by investigating the effects of UV-B radiation at different levels on the natural bioactive compounds: ergothioneine, phytosterols, and polyphenolic compounds in mushroom powders made of brown, white (*Agaricus bisporus*), oyster (*Pleurotus ostreatus*), and shiitake (*Lentinula edodes*) mushrooms, used as dietary supplement ingredients.

MATERIALS AND METHODS

Mushroom Powder Preparation. All mushrooms were grown, harvested, and handled in the Monterey Mushroom, Inc., facility under commercial practices. Fresh mushrooms of brown, white (*Agaricus bisporus*), oyster (*Pleurotus ostreatus*), and shiitake (*Lentinula edodes*) varieties were sliced to 0.3 cm thickness with an Urschel slicer (Valparaiso, IN) and placed in twelve clear plastic bags, with each bag containing approximately 2.26 kg of the sliced material. The bags were labeled with mushroom and assigned treatment type. There were three treatment groups: treatment group one was a control group with no UV-B treatment; in group two (low treatment), the mushrooms were exposed to 80−90 mJ/cm^2 UV-B irradiation for 25 s at 49−54 °C expected to produce 100−200 IU/g (2.5−5 μg/g) vitamin D_2; and in group three (high treatment), the mushrooms were exposed to 2800−2900 mJ/cm^2 UV-B irradiation at 60−66 °C for 10 min to produce 6000−8000 IU/g (150−200 μg/g) vitamin D_2.

For high UV-B radiation treatment, 2.26 kg each of four sliced mushroom samples (white, brown, oyster, and shiitake) were distributed onto the UV line conveyor in a monolayer (1.25 cm thickness) across the width of the conveyor, with 60 cm separating different mushroom type samples. The UV line (Monterey Mushrooms, Inc., proprietary) was set up with commercial UV specifications. The final energy output was measured in mJ/cm^2 with an EIT radiometer UV Power Puck II (Watsonville, CA 95076). After 10 min UV exposure, UV-treated mushrooms were directly placed on trays for dehydration. Low UV-B radiation treatment followed the same technique, with the UV line specifications modified to produce low UV-B levels by removing bulbs and adjusting the conveyor speed to obtain low energy readings. Total exposure time was 25 s, and the treated mushrooms were placed on trays for dehydration. For control samples with no UV treatment, sliced mushroom were directly placed on trays for dehydration.

Trays were loaded into a Harvest Saver (tray dryer) dehydrator (Commercial Dehydrator Systems, Inc.) for 16 h at 93.3 °C, and an empty tray was placed in between each tray with samples to prevent cross contamination. After the dehydration cycle, each sample was placed in a laminated prelabeled bag and sealed. A coffee grinder was used for grinding samples. After each sample, the coffee grinder was washed and dried before the next sample. Mushroom powder samples were placed in laminated bags, sealed and labeled accordingly.

The samples were shipped on dry ice overnight to the USDA laboratories. The samples were stored at −20 °C away from light exposure until the analysis. Moisture content was determined gravimetrically for each mushroom powder sample after drying the mushroom powder in an oven at 110 °C overnight. Each sample of mushroom powder was analyzed in triplicate to test for analytical variability.

Reagents and Materials. Standards of ergosterol (purity >95%), cycloartenol (purity >90%), cholesterol, methimazole, and ergothioneine (purity >99% for all) were purchased from Sigma Aldrich (St. Louis, MO, USA); standards of β-sitosterol, campesterol, stigmasterol, brassicasterol, sitostanol (stigmastanol), lupeol, and campesterol-d_3 (purity >98% for all) were from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Stock solutions were individually prepared from neat materials at concentrations of 1 mg/mL in methanol, acetone, isopropanol, or their mixtures, depending on the compound solubility, and stored at −18 °C. A standard mixture of phytosterols at 10 μg/mL in methanol/acetone was prepared and used as a spiking solution for recovery experiments.

Standards of genistein, daidzein, glycetein, daidzin, and genistin were obtained from LC Laboratories (Woburn, MA). Standards of biochanin A, *trans*-resveratrol, quercetin dehydrate, kaempferol, coumestrol, naringin, formononetin, delphinidin, naringenin, eriodictyol, enterodiol, rutin hydrate, and cyanidin were purchased from Sigma Aldrich (St. Louis, MO, USA). (±)-Equol, matairesinol, (±)-enterolactone, apigenin, hesperetin, luteolin, and piceatannol were from the Cayman Chemical Company (Ann Harbor, MI). Genistein-d_4 was from the CDN Isotopes (Pointe-Claire, Quebec, Canada). All standards were ≥95% purity. A standard mixture of polyphenols at 10 μg/mL in methanol was prepared and used as a spiking solution for recovery experiments. Concentrations were not corrected for the purity of the standards.

All solvents were HPLC grade. Methanol was purchased from Sigma Aldrich (St. Louis, MO, USA), and acetone was from EMD Chemicals (Gibbstown, NJ, USA). Isopropanol was purchased from Honeywell Burdick and Jackson (Muskegon, MI, USA). Certified ACS grade hexane was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Deionized water of 18.2 Ω-cm was prepared with an E-Pure model D4641 from Barnstead/Thermolyne (Dubuque, IA, USA). Formic acid (88% purity) was obtained from Spectrum Quality Products Inc. (New Brunswick, NJ, USA). Potassium hydroxide (KOH) pellets (purity 86.8%) and anhydrous sodium sulfate (purity 99.49%) were obtained from Mallinckrodt, Inc. (Paris, KY, USA). Pyridine (purity 99.8%) was from Sigma-Aldrich. *N*,*O*-Bis-(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TCMS) were purchased from Fisher Scientific (Pittsburgh, PA, USA).

National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 3251 *Serenoa repens* extract was purchased from NIST (Gaithersburg, MD, USA).

Phytosterol Analysis. The sample preparation method was based on that of Laakso (2005)[22] with some modifications. Briefly, mushroom powder samples (100 mg) were spiked with an internal standard (ISTD) of cholesterol (2 μg), then saponified with 2.0 mL of 3 N KOH in methanol by sonication (Branson 5510 Ultrasonicator, Branson Ultrasonics (Danbury, CT, USA)) for 10 min and heating in a water bath at 60 °C for 60 min. Phytosterols were extracted by adding 2 mL of hexane to the samples, vortexing, and then centrifuging to allow separation of the layers. The top organic layer was removed into a clean centrifuge vial, and then the extraction was repeated two more times. The combined hexane extract was then evaporated to dryness at room temperature under nitrogen. Pyridine (25 μL) was added, and the extract was derivatized with 25 μL of BSTFA + 1% TCMS by placing in a water bath at 60 °C for 30 min. After hexane (950 μL) was added, the extract was transferred to an autosampler vial and subjected to gas chromatographic (GC) analysis.

Table 1. Average Retention Times (t_R), Multiple Reaction Monitoring (MRM) Transitions, Collision Energy (CE) for Each Transition, and the Lowest Calibrated Level (LCL)

TMS ether of	t_R (min)	quantifier (m/z)	CE (V)	qualifier (m/z)	CE (V)	LCL ($\mu g\ g^{-1}$)
cholesterol	6.788	368.3 → 159.2	40	368.3 → 145.1	40	ISTD
brassicasterol	6.943	470.4 → 201.3	20	470.4 → 69	20	0.025
ergosterol	7.15	363.2 → 143	40	363.2 → 128	40	0.01
campesterol	7.223	382.3 → 145.1	40	382.3 → 105	40	0.01
stigmasterol	7.328	484.4 → 169.3	40	484.4 → 145	40	0.025
campesterol-d_3	7.422	346.3 → 121.1	10	346.3 → 95.1	10	ISTD
β-sitosterol	7.625	396.3 → 381.3	10	396.3 → 213.1	10	0.05
stigmastanol	7.69	473.3 → 161.2	20	473.3 → 75.1	20	0.01
cycloartenol	8.058	393.3 → 81.2	30	393.3 → 69.2	30	0.01
lupeol	8.14	369.3 → 109.1	10	369.3 → 94.9	10	0.05

The detection and quantification of the trimethylsilyl (TMS) ethers of sterols was performed using an Agilent (Little Falls, DE, USA) 7000B with high sensitivity electron ionization (EI) source triple-quadrupole mass spectrometer (GC–MS/MS). The GC was an Agilent 7890A gas chromatograph operated in low pressure vacuum outlet mode.[23] The GC column was a 15 m × 0.53 mm × 1 μm film thickness SLT-5 MS (Supelco, Bellefonte, PA, USA) connected to a 5 m × 0.18 mm noncoated guard column from Restek (Bellefonte, PA, USA). Ultrahigh purity He was used as a carrier gas at 2 mL min^{-1} constant flow rate as described previously.[24] At the transfer line the GC column was connected to a ∼17 cm × 0.53 mm i.d. piece of phenyl-methyl deactivated guard column (Restek).

The GC oven temperature program was as follows: 70 °C held for 1.5 min, ramped 80 °C min^{-1} to 180 °C, then ramped 40 °C min^{-1} to 250 °C, followed by 70 °C min^{-1} to 290 °C, and held 4.5 min. The total run time was 9.7 min. The transfer line was at 250 °C, and ion source was set at 320 °C. The MS/MS collision gas was nitrogen at 1.5 mL min^{-1}, and the quench gas was He at 2.25 mL min^{-1}. The EI energy was −70 eV, quadrupole temperatures were set at 150 °C, and the solvent delay was 6 min. An Agilent Multimode Inlet (MMI) was operated as a programmable temperature vaporizer (5 μL injection volume) with solvent vent mode of He flow at 55 mL min^{-1}. The MMI temperature program was 70 °C held for 0.31 min (at which point the vent was closed), then ramped to 420 °C at 320 °C min^{-1}, and held at 420 °C for the rest of the GC run.

Each phytosterol individual standard solution at 1000 ng mL^{-1} was derivatized individually with pyridine and BSTFA + TCMS as described above and injected in full scan mode to determine the most intense ions of the corresponding TMS ether. Product ion and collision energy experiments were performed to determine the optimum two product ions, collision energies, and ratios between quantifier and qualifier ions. The method detection limit was defined as a compound's lowest calibrated level. Multiple reaction monitoring (MRM) transitions, collision energy for each transition, average retention times (t_R), and the lowest calibration levels are presented in Table 1. In the finalized MRM method, a dwell time of 2.5 ms and the "wide" MS resolution were used for all transitions. The MRM transitions were monitored in one time segment from 6 to 9 min. Cholesterol was used as a main ISTD, and campesterol-d_3 was used as a backup if needed. Calibration curves were linear from 0.01 to 0.05 to 10 μg/mL for all sterols. Due to the abundance of ergosterol relative to the other sterols in mushroom powder, its calibration curve was from 1 to 50 μg/mL. Separate extraction was performed for ergosterol analysis, the mass of extracted mushroom powder was 20–30 mg, and the extracts were diluted in hexane 10 times prior to GC–MS/MS analysis.

Ergothioneine Analysis. Mushroom powder (100 mg) was placed into a 15 mL polypropylene centrifuge tube, 10 μg of ISTD methimazole was added, and the sample was extracted with 10 mL of 4:1 (v:v) methanol/water by vigorous shaking for 20 min in a shaker, following by centrifugation at 3650 rcf (×g) for 3 min. After centrifugation, 0.1 mL of the extract was transferred to an autosampler vial containing 0.9 mL of 3:7 (v:v) methanol/water, and the extract was subjected to LC–MS/MS analysis.

An HPLC column (Luna C8(2) 3 μm, 100 Å, 100 × 4.6 mm) integrated with a 4 × 3 mm i.d. Security Guard C8 column (Phenomenex Inc., Torrance, CA, USA) was used for separation. An isocratic program of 0.1% formic acid buffer (A) at 30% and MeOH (B) at 70% with a constant flow rate of 0.4 mL min^{-1} was applied with an injection volume of 20 μL, and the column oven was at 30 °C. The total run time was 5 min. The calibration curves were linear from 0.001 to 2.5 μg/mL of ergothioneine with 0.1 μg of the ISTD. The method detection limit was 1 μg/g ergothioneine.

The mass spectrometer was an API 3000 (Applied Biosystems/MDS Sciex, Ontario, Canada) with electrospray ionization (ESI) operated in the positive mode. The ion spray potential was 4500 V, and the source temperature was set at 500 °C. MRMs of m/z 230.2 → 127.1 and m/z 230.2 → 186.1 were used for ergothioneine (t_R = 2.75 min) and MRMs of m/z 114.9 → 57.1 and m/z 114.9 → 74.1 were used for methimazole (t_R = 3.05 min) with dwell times of 250 ms.

Polyphenolic Compound Analysis. Mushroom powder (100 mg) was placed into a 15 mL polypropylene centrifuge tube, 1 μg of an ISTD genistein-d_4 was added, and the sample was extracted with 5 mL of 4:1 (v:v) methanol/water by sonication for 30 min at room temperature. A 0.5 mL aliquot of the extract was filtered through a 0.2 μm PVDF filter and transferred into an autosampler vial, 0.5 mL of DI water was added to each vial, and the extract was subjected to LC–MS/MS analysis.

A Luna C8(2) 3 μm, 100 Å, 100 × 4.6 mm column with a 4 × 3 mm i.d. Security Guard C8 column (Phenomenex Inc.) was used for separation. The binary gradient of 0.2 mM ammonium formate buffer at pH 4.7 (A) and MeOH (B) at a constant flow rate of 0.4 mL min^{-1} was applied with an injection volume of 20 μL. Column oven temperature was at 40 °C. The gradient program started at 70% A held for 1 min, then ramped to 90% B over the course of 7 min, and held for 2 min. Re-equilibration time at 70% A was 4 min. The mass spectrometer with ESI was operated in the negative mode. The detailed information on the LC–MS/MS method used for polyphenols is reported elsewhere.[25] The calibration curves were linear from 0.001 to 2.5 μg/mL. The method detection limit was 0.1 μg/g.

Vitamin D Analysis. Vitamin D analysis was performed as previously described in detail by Byrdwell (2013)[26] using a "dilute-and-shoot" method and analyzed using liquid chromatography quadruple parallel mass spectrometry (MS) with UV detection at 265 nm. Comparison of MS and UV results is a separate study, which will be presented in a future work.

Each mushroom powder sample was prepared in 5 replicate analytical samples, which were each run in triplicate. A set of three calibration standards were bracketed before, between, and after the five samples. Samples that were expected to have low values (control and low treatment) used a low set of standards, consisting of 500, 1000, and 2000 IU. Samples that were expected to have higher values (high treatment) used a higher set of standards, consisting of 1000, 2000, and 5000 IU.

Statistical Analysis. Statistical data analysis was performed with the SAS System for Windows version 9.4 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was conducted to investigate the effects and interactions of treatment and mushroom type on ergosterol, campesterol, ergothioneine, and vitamin D. Dunnett's and Bonferroni tests were used with $p = 0.05$ significance.

RESULTS AND DISCUSSION

Sample Preparation Method Evaluation. Phytosterol recoveries were evaluated using reagent spikes (300 mg of anhydrous Na_2SO_4) and matrix spikes (100 mg of mushroom powder) fortified with 2.5 and 5 mg/g of phytosterol standard mixture. Five replicate samples were prepared for both reagent spikes and matrix spikes at each spiking level (see Table 2).

Table 2. % Recoveries of Phytosterols (and % RSDs)[a]

	reagent spike		matrix spike	
	2.5 mg/g	5 mg/g	2.5 mg/g	5 mg/g
brassicasterol	113 (6)	104 (12)	89 (3)	103 (5)
ergosterol	95 (9)	80 (7)	n/a	n/a
campesterol	107 (5)	104 (7)	107 (20)	113 (7)
stigmasterol	98 (12)	107 (4)	120 (14)	**133** (5)
β-sitosterol	112 (2)	105 (5)	120 (2)	118 (6)
stigmastanol	108 (12)	106 (13)	**121** (9)	119 (15)
cycloartenol	105 (17)	108 (17)	**125** (8)	106 (4)
lupeol	107 (11)	110 (6)	117 (8)	112 (6)

[a] $n = 5$; bold indicates recoveries outside of 70−120%; n/a, not applicable.

Unfortified mushroom powder and reagent blank samples (1 mL of DI water) were analyzed along with the spiked samples. Due to the abundance of ergosterol in mushroom powder, it was impossible to calculate its recoveries for fortified mushroom powder samples, therefore only reagent spike recoveries were evaluated in its case.

Campesterol and ergosterol were measured in mushroom powders used for the fortification experiments. Campesterol recoveries were calculated after subtracting the amounts found in the control mushroom powder from the amounts measured in the fortified mushroom powder. However, this was impossible for ergosterol, as the mushroom powders contained a very high amount of ergosterol.

The recoveries of phytosterols from reagent spike samples were within satisfactory limits of 95−113% (5−17% RSDs) for the 2.5 mg/g spiking level and 80−110% (4−17% RSDs) for the 5 mg/g level. For fortified mushroom powders, however, the recoveries ranged from 89 to 125% (2−20% RSDs) for 2.5 mg/g and 103−133% (4−15% RSDs) for the 5 mg/g spiking level. Cycloartenol and stigmasterol (125 and 133%, respectively) recoveries were outside of satisfactory limits of 70−120%. RSDs for 5 replicate measurements in the recovery experiment were at or below 20%, indicating acceptable method performance.

To estimate the accuracy of measurements (or "trueness") of the developed method, the standard reference material SRM 3251 *Serenoa repens* extract with certified values for campesterol, β-sitosterol, and stigmasterol was analyzed in 5 replicates. Due to high amounts of these sterols in the reference material, only small portions of the material (20−28 mg) were extracted. The measured values were in good agreement with certified values for campesterol and stigmasterol (see Table 3). The average accuracy measured as a percent of measured concentration vs certified concentration was 97% for campesterol and 93% for stigmasterol. For β-sitosterol concentrations measured in SRM 3251, the average accuracy was 124%. β-Sitosterol has the highest concentration in SRM 3251, and even after extraction of small amounts of the reference material, several serial dilutions 1:10 and 1:100 were required to fit its concentration in the calibration curve range. In contrast to the SRM material, β-sitosterol concentrations in mushrooms are very low[9] and were not measured in mushroom samples in our study. RSDs for five parallel measurements of selected phytosterols in SRM 3251 were 11−16%, indicating the method's acceptable repeatability.

Table 3. Determined Phytosterol Concentrations in SRM 3251 *Serenoa repens* Extract

	certified value, mg g^{-1} ± SD	measd value, mg g^{-1} ± SD, $n = 5$	% accuracy (RSD), $n = 5$
campesterol	0.533 ± 0.031	0.519 ± 0.081	97 (16)
β-sitosterol	1.666 ± 0.064	2.065 ± 0.321	124 (16)
stigmasterol	0.247 ± 0.040	0.231 ± 0.037	93 (11)

For evaluating egrothioneine recoveries, the mushroom powder was spiked with ergothioneine standard solution at the levels 0.5, 2, and 5 mg/g ergothioneine. Five replicate samples were prepared as described above for each spiking level. Unfortified mushroom powder and reagent blank samples were analyzed along with the spiked samples, and ergothioneine concentrations for the recovery calculations were adjusted for the amount measured in unfortified mushroom powder. The ergothioneine recoveries were 109 ± 20%, 105 ± 8%, and 119 ± 10% for the spiking levels of 0.5, 2, and 5 mg/g, respectively.

The recoveries of polyphenols from fortified mushroom powder samples were evaluated at spiking levels of 5 and 10 μg/g for five replicates per level. Unfortified mushroom powder and reagent blank samples were analyzed along with the spiked samples. No polyphenols were detected in the reagent blank and unfortified mushroom powder samples used in the recovery study. The recoveries of polyphenols were satisfactory (70−120%) with the exception of naringin (63%) and matairesinol (65%) at the 5 μg/g spiking level, and enterolactone (123%) at the 10 μg/g spiking level (see Table 4).

Phytosterols. Only ergosterol and campesterol were present for determination in the mushroom powder samples. Matrix spike sample recoveries were 107−113%, and precision measurement of duplicate samples was 3−20%.

Ergosterol concentrations were 2.4−4.0 mg/g dw (shiitake), 2.9−4.5 mg/g dw (oyster), 4.6−6.2 mg/g dw (brown), and 4.6−5.2 mg/g dw (white) mushroom powder samples (Figure 1). A Bonferroni test showed significantly higher ($p < 0.05$) ergosterol levels in brown and white mushroom powder than in the oyster and shiitake (See Figure 2). This is consistent with the recent findings by Villares et al. (2014)[8] reporting ergosterol concentrations in *A. bisporus* (white and brown) mushrooms (6.42 mg/g dry wt) being significantly greater ($p < 0.05$) than in *L. edodes* (shiitake) (3.64 mg/g dw) and *P. ostreatus* (oyster) (3.31 mg/g dw).

When examining the effect of UV-B treatment among different types of mushroom powder samples, shiitake and oyster mushroom powders had significantly lower concentrations of ergosterol in high UV-B treatment samples compared to control. For brown mushroom powders, samples from high UV-B treatments had significantly lower concen-

Table 4. % Average Recoveries (and % RSDs) of Polyphenolic Compounds in Spiked Mushroom Powder[a]

	spiking levels	
compound	5 μg/g	10 μg/g
naringin	**63** (12)	107 (20)
daidzein	104 (13)	89 (10)
daidzin	79 (15)	78 (20)
genistein	77 (13)	91 (13)
glycitein + biochanin A	113 (9)	118 (16)
genistin	119 (15)	70 (19)
resveratrol	78 (16)	85 (**21**)
quercetin	117 (15)	100 (15)
kaempferol	95 (13)	98 (12)
coumestrol	97 (9)	92 (18)
naringenin	115 (14)	110 (13)
hesperetin	118 (15)	95 (5)
luteolin	117 (**21**)	116 (11)
apigenin	109 (19)	93 (16)
matairesinol	**65** (19)	110 (17)
enterolactone	72 (9)	**123** (14)
equol	83 (13)	120 (4)
eriodictyol	83 (16)	78 (**23**)
enterodiol	118 (11)	96 (15)
rutin	96 (16)	111 (12)
formonetin	89 (15)	89 (19)
cyanidin	80 (10)	102 (16)
delphinidin	**127** (10)	101 (20)
piceatannol	104 (12)	77 (11)

[a] $n = 5$; bold indicates recoveries outside of 70−120% and RSDs >20%.

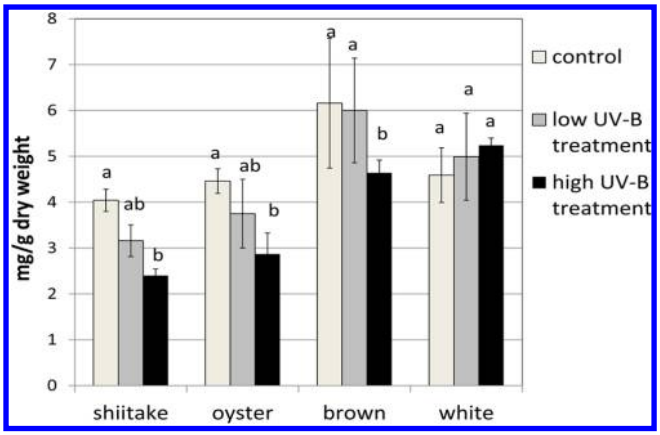

Figure 1. Ergosterol average concentrations (mg/g dry weight) in different types of mushroom powder in control and different treatment conditions; error bars represent standard deviations for $n = 3$. Any two values with no letter in common are significantly ($p < 0.05$) different according to the Bonferroni test within any specific mushroom type.

trations of ergosterol compared to low UV-B treatment and control (see Figure 1).

No statistical difference in ergosterol concentrations was observed for white mushroom powder samples exposed to different treatments. Simon et al. (2011)[27] observed the same effect when ergosterol concentrations remained stable in the same type of mushrooms (*Agaticus bisporus)* exposed to UV-B light. These observations may warrant future studies to better understand a mechanism of ergosterol conversion to vitamin D_2 in different types of mushrooms exposed to UV-B radiation.

Only a small portion of ergosterol converts to vitamin D under UV-B treatment, where ergosterol concentrations in mushrooms are in mg/g levels, and the amounts of formed vitamin D are in μg levels, therefore mushroom powder used as a dietary supplement ingredient provides high levels of ergosterol. According to Phillips et al. (2011),[9] the potential contribution of ergosterol by fresh mushrooms is 0.27−0.86 mg/g. Nuts and seeds are regarded as foods providing the highest amounts of ergosterol: 0.95−4.01 mg/g.[28] In comparison, mushroom powder made of brown mushrooms in our study provides up to 6 mg/g of ergosterol.

Besides ergosterol, campesterol was measured in mushroom powder samples in our study in concentrations of 31−41 μg/g dw (shiitake), 15−20 μg/g dw (oyster), 36−42 μg/g dw (brown), and 38−43 μg/g dw (white) mushroom powder samples (Figure 3). Campesterol concentrations were significantly lower ($p < 0.05$) in oyster mushroom powder compared to three other types (Figure 4). Shiitake mushroom powder had significantly lower concentrations of campesterol in high UV-B treated samples compared to low UV-B treated samples (Figure 3). This is an interesting finding, as we are not aware of any published studies to date reporting the effects of UV-B radiation on campesterol concentrations.

Compared to other studies, concentrations of campesterol in our study were 10 times lower compared to concentrations of campesterol found by Phillips et al. (2011)[9] in morel mushrooms (12.3−45.4 μg/g fresh weight). However, they were comparable with campesterol concentrations measured in *Tuber* fermentation mycelia (15−24 μg/g dw) by Tang et al. (2012).[29] Also, brassicasterol was measured in morel mushrooms[9] and *Tuber* fermentation mycelia,[29] but was not detected in mushroom powder in our study.

Polyphenolic Compounds. No individual polyphenolic compounds were detected in mushroom powder samples in our study (method detection limit 0.1 μg/g). In contrast, quercetin was measured in concentrations 9.64−66.74 μg/g dry weight in wild edible mushrooms from China by other researchers.[18] Another study analyzed phenolic compounds in edible and medicinal mushrooms from Korea and measured naringin (not detected (nd)−38 μg/g), resveratrol (nd−12 μg/g), quercetin (nd−52 μg/g), naringenin (nd−36 μg/g), kaempferol (nd−53 μg/g), hesperetin (nd−3 μg/g), formononetin (nd−1 μg/g), and biochanin A (nd−3 μg/g).[19] Similar to our study, rutin was not detected in mushrooms from Korea. Another study found no flavonoids or lignans in the cultivated mushrooms (white and brown *A. bisporus*, *L. edodes*, and *P. zostreatus*).[30]

Previously published studies have reported that UV-B irradiation increased concentrations of selected polyphenolic compounds, such as flavonoids and flavonols in tomatoes[20] and apples,[21] however the measured amounts were in low mg/g levels, while polyphenolic compounds reported in mushrooms are usually reported in the low μg/g range. According to Kalac (2013),[1] wild and cultivated mushrooms as well as several specific mushroom species may greatly differ in polyphenolics composition and concentrations. Polyphenolic compounds easily degrade when exposed to light, elevated temperatures, and pH changes.[31,32]As it was described by other researchers, elevated temperatures, for example, boiling mushrooms in water, resulted in decreased amounts of total polyphenols and ergothioneine.[33] A recent study indicated that up to 73% of flavonoids were lost during freeze-drying of a perennial herb *Centella asiatica*.[34] It is unclear if polyphenolic compounds were not detected in the mushroom powder samples due to

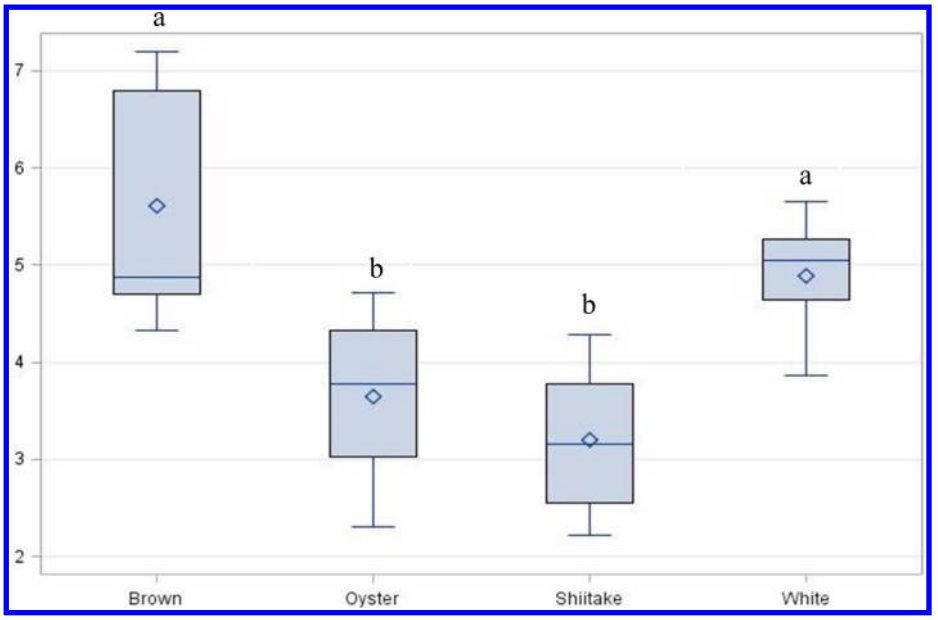

Figure 2. Distribution of ergosterol mean concentrations (mg/g dry weight) among different mushroom powder types. Any two values with no letter in common are significantly ($p < 0.05$) different according to the Bonferroni test.

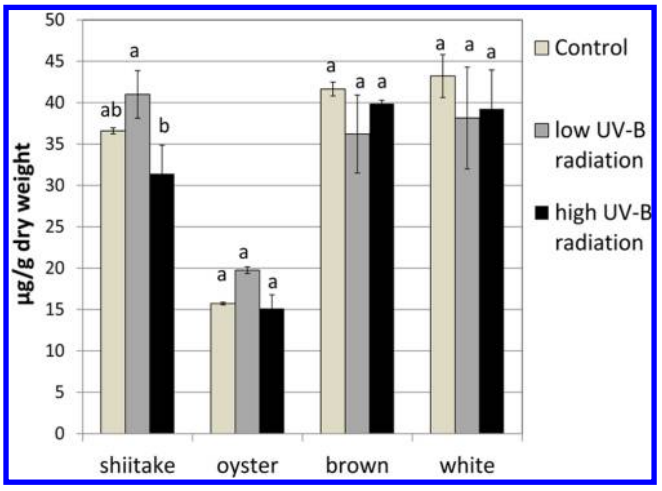

Figure 3. Campesterol average concentrations (μg/g dry weight) in different types of mushroom powder in control and different treatment conditions; error bars represent standard deviations for $n = 3$. Any two values with no letter in common are significantly ($p < 0.05$) different according to the Bonferroni test within any specific mushroom type.

compositional differences in mushroom types, or degradation during the drying process to produce mushroom powder.

Ergothioneione. Ergothioneine recoveries in matrix spike samples were 67−115%, and precision for replicate samples was 0.1−8%. Ergothioneine concentrations found in mushroom powder samples were 0.37−0.48 mg/g dw (brown), 0.81−0.92 mg/g dw (white), 7.2−7.9 mg/g dw (shiitake), and 9.7−10.4 mg/g dw (oyster) (Figure 5). Ergothioneine average concentrations were significantly greater in oyster mushroom powders compared to shiitake, and both types had significantly higher concentrations compared to brown and white (Figure 5). For comparison, Nguyen et al. (2012) found 2 mg/g dw ergothioneine in the cultivated mushroom *Flammulina velutipes*.[33] Other researchers measured 0.21 mg/g, 1.98 mg/g, and 2.59 mg/g dw of ergothioneine in white, shiitake, and oyster mushrooms, respectively.[17] While these amounts are approximately four times lower than amounts of ergothioneine measured in our work, they follow the same trend with oyster mushrooms having the highest amount of ergothioneine, followed by shiitake and white mushrooms. Ey et al. (2007)[16] measured 119 μg/g wet weight of ergothioneine in oyster mushrooms (*Pleurotus ostretus*). Assuming the moisture content of these mushroom was ≈90%, this corresponds to 12 mg/g dw of ergothioneine, which is similar to the 9.7−10.4 mg/g dw of ergothioneine measured in our study. Ergothioneine concentrations were not appreciably affected by UV-B radiation for all mushroom types, except shiitake, for which concentrations of ergothioneine decreased significantly from control to low and high UV-B treatments (Figure 5). This is an important finding, as to our knowledge this is the first study investigating ergothioneine concentration changes in UV-B exposed mushrooms.

Mushrooms can serve as an excellent source of the naturally occurring antioxidant ergothioneine. Shiitake and oyster mushroom powders in this study had the highest amounts of ergothioneine, and only 0.5−1 g of these mushroom powder supplements is needed to provide 4−5 mg of ergothioneine, compared to the need for a ≈70 g serving of fresh mushrooms to receive a similar amount of ergothioneine.

Vitamin D. Vitamin D average concentrations ($n = 5$) and standard deviations, expressed in IU/g, are given in Table 5. % precision for replicate samples ($n = 5$) ranged from 0.53 to 3.14%. No vitamin D was measured in control samples of oyster and shiitake mushroom powder. For statistical analysis, zeroes were used for these samples. In contrast, control samples of brown and white mushroom powder had 179 ± 4 (brown) and 179 ± 5 (white) IU/g of vitamin D. For low UV-B treatment, which was expected to increase vitamin D levels to 100−200 IU/g, vitamin D measured value was close to expected only for shiitake mushroom powder (217 ± 7 IU/g). Vitamin D concentrations measured in brown (1942 ± 30 IU/g) and white (2156 ± 43 IU/g) mushroom powder exposed to low UV-B radiation were statistically greater than expected. For high UV-B treatment, expected to increase vitamin D levels to 6000−8000 IU/g, the vitamin D concentration measured in

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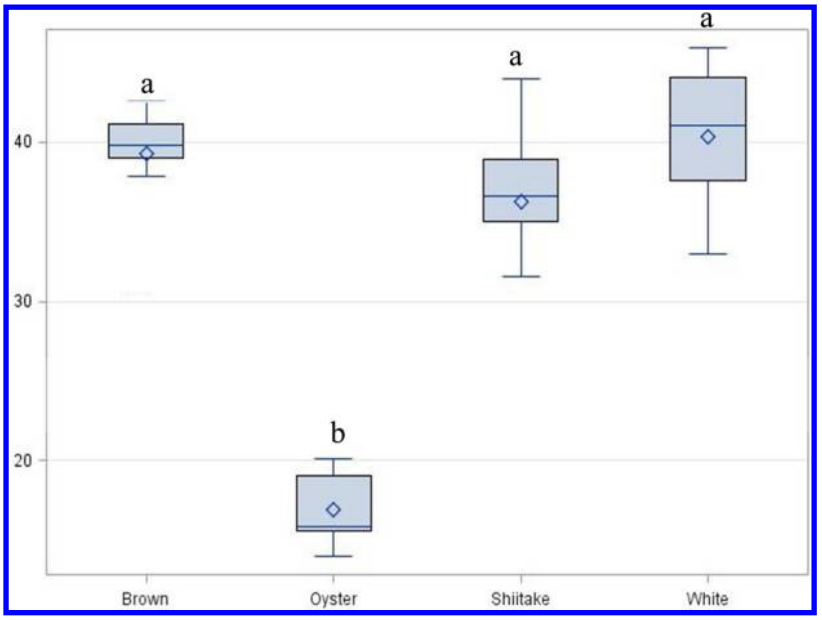

Figure 4. Distribution of campesterol mean concentrations (μg/g dry weight) among different mushroom powder types. Any two values with no letter in common are significantly ($p < 0.05$) different according to the Bonferroni test.

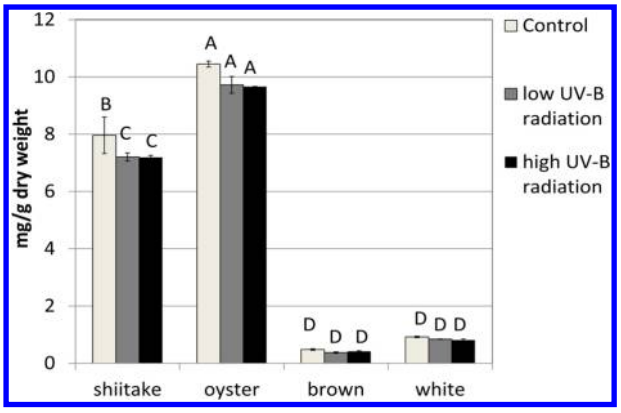

Figure 5. Ergothioneine average concentrations (mg/g dry weight) in different types of mushroom powder in controls and different treatment conditions, error bars represent standard deviations for $n = 3$. Any two values with no letter in common are significantly ($p < 0.05$) different according to the Bonferroni test.

Table 5. Vitamin D Average Concentrations and Standard Deviations ($n = 5$), IU/g in Mushroom Powder Samples[a]

	shiitake	oyster	brown	white
control	nd[b] (**I**)	nd (**I**)	179 ± 4 (**H**)	179 ± 5 (**H**)
low UV-B treatment (expected 100–200 IU/g)	217 ± 7 (**H**)	1245 ± 9 (**F**)	1942 ± 30 (**E**)	2156 ± 43 (**D**)
high UV-B treatment (expected 6000–8000 IU/g)	1036 ± 13 (**G**)	4411 ± 23 (**C**)	6292 ± 109 (**A**)	4739 ± 61 (**B**)

[a]Bold letters in parentheses represent level of statistical difference. Any of the twelve values with no letter in common are significantly different ($p < 0.05$) according to the Bonferonni test. [b]Not detected.

brown mushroom powder (6292 ± 109 IU/g) was at the expected level. Concentrations of vitamin D for oyster (4441 ± 23 IU/g), white (4739 ± 61 IU/g), and shiitake (1036 ± 13 IU/g) mushrooms were statistically lower than expected.

It is interesting to note that shiitake mushroom powder had the lowest levels of ergosterol (significantly lower than in other mushroom powder samples), and the lowest levels of vitamin D were produced in these samples.

This study investigated effects of UV-B radiation on the bioactive natural compounds in different types of mushroom powders used as dietary supplement ingredients. Mushroom antioxidant, ergothioneine, concentrations were 0.37–0.48 mg/g dw (brown), 0.81–0.92 mg/g dw (white), 7.2–7.9 mg/g dw (shiitake), and 9.7–10.4 mg/g dw (oyster) mushroom powder samples, and were mostly not affected by UV-B radiation. Polyphenolic compounds were not detected. Mushrooms' most abundant phytosterol, ergosterol, concentrations were highest for brown (2.9–4.5 mg/g dw) followed by white (4.6–5.2 mg/g dw), oyster (2.9–4.5 mg/g dw), and shiitake (2.4–4.0 mg/g dw) mushroom powder samples. Ergosterol concentrations were significantly higher in brown and white mushroom powders compared to oyster and shiitake, and significantly decreased with the increased level of UV-B treatment for all mushroom powder types, except for white. Campesterol concentrations were greatest for white (38–43 μg/g dw), followed by brown (36–42 μg/g dw), shiitake (31.4–41.0 μg/g dw), and oyster (15.1–19.8 μg/g dw), and were significantly lower in oyster mushroom powder samples compared to the other three types. Campesterol concentrations significantly decreased in shiitake mushroom powder in high UV-B treated samples compared to low UV-B treated samples. These results provide some new information on UV-B radiation effects on the important natural bioactive compounds in mushrooms, and suggest that mushroom powder dietary supplements not only provide vitamin D but also may be a good source of phytosterols and ergothioneine.

AUTHOR INFORMATION

Corresponding Author

*E-mail: yelena.sapozhnikova@ars.usda.gov.

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